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DRUG DISCOVERY

Antibacterial activity of certain medicinal plants against opportunistic pathogenic bacteria

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ABSTRACT

A study was conducted to check the efficacy of certain herbal medicinal plants against opportunistic pathogenic bacteria. Plant extracts of *Allium sativum, Zingiber officinale, Curcuma longa, Syzigium aromaticum, Trigonella foenam graeaum* were extracted out by solvent extraction method .Two solvents were employed for the extraction process by Soxhlet apparatus including petroleum ether (Method A) and Methanol (Method B). The comparative antibacterial efficacy test was conducted for obtained plant extracts with Positive Control of Streptomycin(100µg ml^{-1}) as well Ciprofloxacin (100µg ml^{-1}) and Negative control of Petroleum Ether. Opportunistic pathogenic bacteria used for antibacterial study included*Escherichia coli, Enterobacter aerogenes, Proteus vulgarius, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexnerii*. Disc diffusion assay was carried out for the selected pathogens. Among the extract obtained with Petroleum Ether, *Allium sativum, Zingiber officinale and Syzigium aromaticum* showed antimicrobial activity against all test organisms and their zone of inhibition ranged from 20 to 25 mm, 16 to 23 mm, and 13 to 33 mm respectively.*Curcuma longa* showed antimicrobial activity against *Escherichia coli, Enterobacter aerogenes and Shigella flexnerii* and their zones of inhibition ranged from 12 to 20 mm.The results obtained in this study indicate a considerable difference in antimicrobial activity between extract obtained with method A and B, the petroleum ether extract (Method A) being more effective than the other (Method B).

Key words: Herbal medicinal plants, Plant extract, Solvent Extraction, Antibacterial activity, Disc Diffusion assay

Abbreviations: R.P.M-Rotations per Minute.

Evolution:
Evolution is the change in the inheritedcharacteristics of biologicalpopulations over successive generations. Evolutionary processes give rise to diversity at every level of biological organisation, including species, individual organisms and molecules such as DNA and proteins.

Antibiotics:
Antibiotics, also known as antibacterials, are types of medications that destroy or slow down the growth of bacteria. The Greek word *anti* means "against", and the Greek word *bios* means "life".

1. INTRODUCTION

Dependence on plants as the source of medicine is prevalent in developing countries where traditional medicine plays a major role in the health care (John et al. 2001). The rural population in different parts of the world is more disposed to traditional ways of treatment because of their easy availability and lower cost. A number of studies have been made on antimicrobial activities and identification of active compounds with antibiotic property. However, information is not available on the antimicrobial activity of plants or their parts routinely used. A number of antibiotics derived from microorganisms are currently in use to treat a variety of infectious diseases. Among them many have a limited antimicrobial spectrum due to frequent evolution of drug resistant mutant strains (Gehlot et al. 1998). India has about 45,000 plant species; medicinal properties have been assigned to several thousands, about 2000 are found in literature, indigenous system commonly employ about 500-700 (Bhusan et al. 2004).

2. SCOPE OF THE STUDY

Anattempt was made to study efficacy of five different plant part extract named *Allium sativum, Zingiber officinale, Curcuma longa, Syzigium aromaticum* and *Trigonella foenam graeaum* against the opportunisticpathogenic bacteria named *Escherichia coli, Enterobacter aerogenes, Proteus vulgarius, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella flexnerii.*

2.1. Materials

The plants used in this study viz. *Allium sativum* (bulbs, 10.0 gms), *Zingiber officinale* (Rhizomes, 10.0 gms), *Syzigium aromaticum* (Flower buds, 15.0 gms), *Curcuma longa* (Rhizomes, 20.0 gms), *Trigonella foenum graeaum* (Seeds, 15.0 gms) were collected from the garden of ``Naturopathy centre'', Gujarat Vidyapith, Sadra, Gujarat, India. The test organisms used in this study included*Escherichia coli, Enterobacter aerogenes, Proteus vulgarius, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexnerii*and were collected from J & J College of science, Nadiad, Gujarat, India. Pure cultures of the bacterial species were maintained at 4^0C on Bacteriostatic Agar and

Figure 1
Soxhlet Extractor for extraction process of *Curcuma longa*

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Table 1 Effect of plant on experimental organisms

Plant Extracts	Part Used	Effective diameter of zone of inhibition after 24 h (mm)					
		Escherichia Coli	*Enterobacter aerogenes*	*Pseudomonas aeruginosa*	*Proteus Vulgarius*	*Shigella flexnerii*	*Salmonella Typhi*
Allium sativum	Bulbs	25.0	24.0	23.0	26.0	20.0	24.0
Zingiber officinale	Rhizome	20.0	17.0	23.0	16.0	20.0	16.0
Syzigium aromaticum	Flower Buds	12.0	33.0	20.0	14.0	13.0	20.0
Curcuma longa	Rhizome	20.0	18.0	0.0	0.0	12.0	0.0
Trigonella foenam graeaum	Seeds	13.0	0.0	0.0	15.0	10.0	15.0
ANTIBIOTICS: Streptomycin (100µg ml^{-1})	-	16.0	25.0	Not Used	24.0	23.0	20.0
ANTIBIOTICS: Ciprofloxacin (100µg ml^{-1})	-	Not Used	Not used	26.0	Not used	Not used	Not Used
Pure petroleum Ether	-	0.0	0.0	0.0	0.0	0.0	0.0

Figure 1
Inhibitory Effect of *Allium sativum* on *Shigella flexnerii*

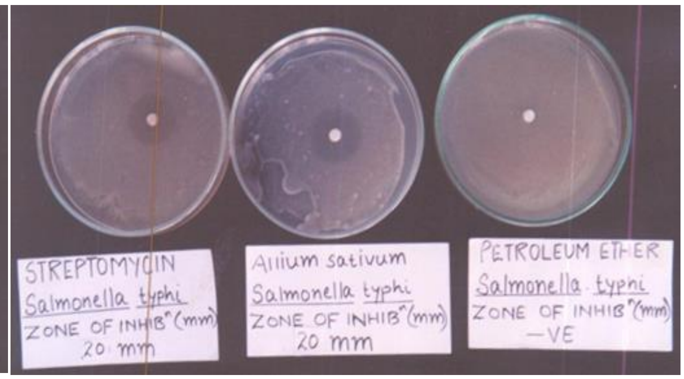

Figure 2
Inhibitory Effect of *Allium sativum* on *Salmonella typhi*

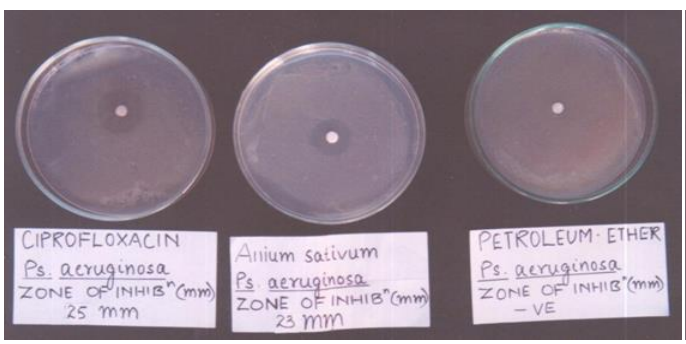

Figure 3
Inhibitory Effect of *Allium sativum* on *Pseudomonas aeruginosa*

Figure 4
Inhibitory Effect of *Allium sativum* on *Proteus vulgarius*

sub-cultured once in every month. For antimicrobial testing, cells were grown overnight in Nutrient Broth at 37^{0}C for 24 hours.

2.2. Methodology

Two method of extraction were employed. In method A, a known amount of crushed parts of *Allium sativum, Zingiber officinale, Syzigium aromaticum, Curcuma longa* were extracted separately at 45^{0}C with Petroleum ether using a Soxhlet extractor (Fig.1). In method B, dried powder of seeds of *Trigonella foenum graeaum* was extracted with methanol at 4^{0}C using centrifugation at 5000 R.P.M. The yields of method A & B were approximately 1.0 and 1.5 ml respectively, which were concentrated to dryness using hot air oven (80^{0}C) for 1 hour. For antibacterial testing, yield of each extract was directly used. Disc diffusion assays were carried out using the method of Wilkins et al (1972). Briefly, a lawn of microorganisms was prepared by pipetting by evenly spreading 50 µl of overnight cultures (10^7 to 10^8 CFU/ml) onto agar set in petridishes. Bacteriostatic Agar was used for bacterial strains. A 5.0 mm sterile paper disc was placed at the centre of this agar plate to which the direct yield of each extract of particular plant was added separately. The plates were inverted and incubated for 24 hours at 37^{0}C after which the diameter of the zone of inhibition around the disc was measured. Control experiments were performed where only equivalent volume of antibiotics and pure petroleum ether without added test compound were applied to the paper discs (Chandet al. 1994). Pure petroleum ether was used as negative while streptomycin (100µg ml^{-1}) and ciprofloxacin discs (100µg ml^{-1}) wereused as positive control.

3. RESULTS

The results of the disc diffusion testing of plant extract are listed in Table 1. The Petroleum ether negative control showed no inhibitory effect. The positive control showed inhibition zones ranging from 16 to 26 mm (Streptomycin and Ciprofloxacin) against Gram-negative opportunistic pathogens. Among the extract obtained with method A, *Allium sativum, Zingiber officinale and Syzigium aromaticum* showed antimicrobial activity against all test organisms and their zone of inhibition ranged from 20 to 25 mm, 16 to 23

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Table 2 Plant Extracts and their chemical constituents

PLANT EXTRACTS	CHEMICAL CONSTITUENTS
Allium sativum	Allicin
Zingiber officinale	Volatile Oil: zingiberine, sesquiterpine, zingiberol, bissaboline
Syzigium aromaticum	Volatile Oil: eugenol, acetyl, eugenol, sesquiterpenes, caryophellenes
Curcuma longa	Volatile Oil: phellendrend, subieneborenol, cineole, ketone tumerone
Trigonella foenam graeaum	Alkaloids, choline, essential oil, saponin

mm, and 13 to 33 mm respectively. It was interesting to note that the petroleum ether extract of *Syzigium aromaticum* exhibited antimicrobial activity against all test pathogens. It showed the highest inhibitory activity against *Enterobacter aerogenes*, exhibiting a zone of inhibition of 33.0 mm. *Curcuma longa* showed antimicrobial activity with petroleum ether extract against *Escherichia coli, Enterobacter aerogenes and Shigella flexnerii* and their zones of inhibition ranged from 12 to 20 mm. No activity was found with petroleum ether extract of *Curcuma longa* against *Proteus vulgarius, Pseudomonas aeruginosa* and *Salmonella typhi*. Among the extract obtained by method B, *Trigonella foenum graecum* showed antimicrobial activity against *E.coli, P. Vulgarius, S. Flexnerii, Salmonella typhi and* their zone of inhibition ranged from 10 to 15 mm. No activity was found with methanol extract of *Trigonella foenum graecum* against *Enterobacter aerogenes and Pseudomonas aeruginosa.* The petroleum ether extracts of *Curcuma longa* and methanol extract of *Trigonella foenum graecum* exhibited weak antimicrobial action when compared to other extracts. Table 3 illustrates Pictures details and Fig. 2 to 5 reveals inhibitory effect of *Allium sativum*on different pathogenic bacteria. The results obtained in this study indicate a considerable difference in antimicrobial activity between extract obtained with method A and B, the petroleum extract (Method A) being more effective than the other (Method B).

Hydrophilic:
Hydrophilic term defined as water loving compounds which have an affinity to water and are usually charged or have polar side groups to their structure that will attract water.

Lipophilicity:
Lipophilicity refers to the ability of a chemical compound to dissolve in fats, oils, lipids, and non-polar solvents such as hexane or toluene. These non-polar solvents are themselves lipophilic — the axiom that *like dissolves like* generally holds true.

4. DISCUSSION

The inhibitory effect of petroleum ether extract of *Allium sativum* may be due to presence of Allicin. Allicin is produced from water soluble allin by action of allin lyase (Shah et al.1995; Arumugun et al. 1993) during crushing and distillation of bulbs of *Allium sativum*. Petroleum ether extract of *Zingiber officinale and Syzigium aromaticum* controlled all the test pathogens. Zingiberene, Zingiberol and Bissaboline are the three major biological substances in petroleum ether extract of *Zingiber officinale*, which may be responsible for the biological activity of *Zingiber officinale* (Shah et al.1995; Arumugun et al. 1993). *Syzigium aromaticum* may contains Eugenol, Acetyl eugenol, Caryophellenes and a certain degree of lipophilicity might determine toxicity against test organisms (Shah et al.1995; Arumugun et al. 1993). *Pseudomonas aeruginosa* causes a number of diseases such as burn infection, Otitis media, ear infection and conjunctivitis as an opportunistic pathogen. Even higher doses of antibiotics fail to control these diseases due to frequent evolution of drug resistant mutant strains of *Pseudomonas aeruginosa.* In such cases the active principles of *Allium sativum, Zingiber officinale and Syzigium aromaticum* may prove to be useful. The activity of all extracts was more pronounced against *E.coli and Shigella flexnerii* than others. The antibacterial compounds of the plants assayed are not well known. However, the presence of following compound (Table 2) might determine toxicity by the interaction with the cell wall and cell membrane constituents and their arrangement. Gram negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solute, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Daltons (Nokaido et al, 1985). Thus the chemical constituent showed in Table 2, might possess the capability to pass the cell wall and cell membrane due to which they exhibit antimicrobial activity.

5. CONCLUSION

Method A proved to be more efficient in extracting the active antibiotic substances. The best results were obtained with *Allium sativum* extract against all tested organisms but the other plants extract also proved to be moderately toxic against the test organisms. In case of *Syzigium aromaticum, the* extract showed the highest inhibitory effect (33.0 mm) against *Enterobacter aerogenes*. In a comparative study between plant extract and antibiotics, *Allium sativum, Zingiber officinale and Syzigium aromaticum* exhibited better activity than standard antibiotics, while *Curcuma longa and Trigonella foenam graecum* showed poor inhibitory effect than standard antibiotics. Further studies on isolation of active principle and their assay for antibiotic properties will prove useful.

SUMMARY OF RESEARCH

The study highlights the antibacterial efficacy for different herbal medicinal plants includes *Allium sativum, Zingiber officinale, Curcuma longa, Syzigium aromaticum, Trigonella foenam graeaum* against opportunistic pathogenicbacteriaincludes *Escherichia coli, Enterobacter aerogenes, Proteus vulgarius, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexnerii*., The knowledge regarding presence of antibacterial substances in herbal medicinal plants is useful to determine the degree of alternative source to formulate ayurvedic drugs.

FUTURE ISSUES

Most of the plants, which are medicinally important, are available in India but their significance and method of utilization is not known, thus it becomes necessary to take special effort to publicize these neglected plants of our backyards. The undesirable side effects of some of the present day antibiotics and the resistance developed by some bacteria to modern antibiotic therapy call for the discovery of new antibacterial substances which could overcome these two therapeutic limitations. More detailed investigations are required on this aspect and if significant results are achieved, the pharmaceutical industries will prosper and benefit by nature's own fit. From the finding, it deduced that the selected herbal medicinal plants *Allium sativum, Zingiber officinale, Curcuma longa, Syzigium aromaticum, Trigonella foenam graeaum* against opportunistic pathogenic bacteria includes*Escherichia coli, Enterobacter aerogenes, Proteus vulgarius, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexnerii* exhibits the antibacterial activity, suggesting that the data can be employed for commercial production to boast good quality of manufacturing of herbal drugs.

DISCLOSURE STATEMENT

There was the financial provision from the organization for this research listed below: Gujarat Vidyapith, Sadra, Gujarat, India.

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